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Serial Endothelial Cell Count of Donor Corneal Buttons in Optisol-GS

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Abstract

Optisol–GS storage medium allows donor corneas to remain viable until they are transplanted to cornea-blind recipients. In order to remain viable, the corneal endothelial cell density (ECD) must remain above 2 200 cells per mm^2. The objective of this study is to determine how long donor corneas may remain viable in Optisol-GS based on their ECD. Although our findings show that the ECD of corneas stored in Optisol-GS decreased steadily, it remained above the critical level of 2 200 cells per mm^2 for up to 9 days, suggesting that corneal buttons may remain viable for at least this amount of time.

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Keywords: Corneal endothelial cell density; corneal endothelial cell loss; corneal preservation; corneal transplantation; donor cornea viability; Optisol-GS corneal storage medium; specular microscopy

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Nomenclature

ECD	endothelial cell density
mm	millimeter
d	day
min	minute
yr	year

1. Introduction

Based on a survey conducted by the WHO in 2002, there are 37 million blind individuals. Of these, 5.1 % are corneal in etiology[1]. Keratoplasty, both penetrating and lamellar, are procedures that have enabled cornea-blind individuals to improve or regain their vision. In this procedure, diseased corneal tissue is removed and replaced by cadaveric donor corneal material. These donor corneas are harvested from recently deceased individuals and transferred to corneal storage medium until transplanted to a recipient.

Endothelial cells line the inner surface of the cornea, and are responsible for corneal nutrition and clarity. These cells do not divide and therefore, have a finite population. As a general rule, the lower the endothelial cell density (ECD), the less viable the cornea is[2,3].

Under normal conditions, the rate of endothelial cell loss is estimated to be 0.6 % · yr^{-1} [4]. In a 10-year cohort study by Ing et al[3], the annual rate of endothelial cell loss 3 yr to 5 yr post-keratoplasty was 4.2 % · yr^{-1}, seven times the normal rate. This study recommends the need for donor corneas to have a satisfactory ECD prior to keratoplasty to minimize the risk of endothelial failure. At the Santa Lucia International Eye Bank of Manila / Eye Bank Foundation of the Philippines (SLIEBM/EBFP), the critical level of ECD is set at 2 200 cells · mm^{-2}. Corneas that have ECDs below this level are deemed not suitable for optical keratoplasty.

Optisol-GS (Chiron Ophthalmics, Irvine, CA, USA) is a widely used corneal storage medium that prolongs corneal viability following harvest. It contains 2.5 % chondroitin sulfate, 1 % dextran, vitamins and precursors of adenosine triphosphate[5]. Based on analyses of long-term results of penetrating keratoplasty, it has been estimated that corneas stored in Optisol-GS may remain viable for up to 14 d to 21 d[6,7]. However the maximum length of time of useful storage has not yet been determined. The objective of this study is to monitor on a daily basis the ECD of corneas preserved in Optisol–GS and determine the rate of ECD loss. This information may help determine how long these corneas may remain viable in this preservative solution.

2. Materials and methods

Twenty-one human corneas from eleven Filipino donors (mean age = 57.62 yr ± 12.87 yr; range = 33 yr to 75 yr) were evaluated at the SLIEBM/EBFP. All donor tissues met the inclusion and exclusion criteria for human corneal transplantation (Table 1 and Table 2). After certifying that the donor had previously consented to donating his/her corneas and/or obtaining consent from the donors' families, corneal tissues were harvested then stored in glass vials with Optisol-GS at 4 °C.

Endothelial cell count was measured daily using a Konan Specular Microscopic Keratoanalyzer (Hyogo, Japan) from d 0 (day of harvest) until d 10. The corneoscleral buttons were brought to room temperature for 30 min before examinations were performed. Once the specimens were sufficiently warmed, they were photographed immediately for measurement of ECD. The corneas were re-refrigerated immediately after each assessment. The readings were performed in triplicate, averaged and subjected to regression analysis.

Table 1. Donor cornea inclusion criteria

No.	Inclusion Criteria
1.	Donors must be deceased individuals of any age with a known cause of death.
2.	Proper consent from the donor and/or relatives must have been obtained.
3.	Corneas must have been harvested within 12 hours from time of death.
4.	Corneas must be undamaged or minimally damaged.
5.	Eyes must not have undergone ocular surgery.

Table 2. Donor cornea exclusion criteria

No.	Exclusion Criteria
1.	CMV Infection
2.	Congenital Rubella
3.	Conjunctivitis
4.	Creutzfeld-Jacob Disease
5.	Death from CNS disease of unknown etiology
6.	Death of unknown cause
7.	Dementia
8.	Encephalitis
9.	Hepatitis (Active)
10.	High risk for blood-borne diseases
11.	HIV Infection
12.	Hodgkin's Disease
13.	Leukemia
14.	Lymphosarcoma
15.	Multifocal Leukoencephalopathy
16.	Pneumonia
17.	Post-refractive surgery (e.g., LASIK, EPILASIK) and/or intraocular surgery
18.	Pulmonary TB (active)
19.	Rabies
20.	Reye's Syndrome
21.	Septicemia
22.	Subacute Sclerosing Panencephalitis
23.	Syphilis (Active)

3. Result and discussion

Serial specular microscopy revealed that Optisol-GS preserved corneas remained clear until the 9th day post-harvest. The endothelium began to be unreadable on the 10^{th} post-harvest day. The ECD at harvest was 3 134.78 cells per mm^2 ± 199.14 cells per mm^2 and 2 324.76 cells per mm^2 ± 344.38 cells per mm^2 on d 9. The data shows that endothelial cell loss decreased in a linear fashion. By regression analysis, the rate of endothelial cell loss was 93.08 cells per mm^2 per day of storage. Pearson's correlation coefficient showed a strong negative correlation between ECD and the length of storage time ($r = -0.98$) (Figure 1).

The corneal endothelium is essential for the maintenance of normal corneal deturgescence. For donor corneas to remain viable after harvest, they must be preserved in storage media such as Optisol-GS. ECD serves as a measurement of the functional status of endothelial cells and can serve as a gauge of corneal button viability[2,3]. A critical ECD of 2 200 cells per mm^2 is necessary to maintain corneal qualities necessary for successful transplantation.

This study focused on the rate of endothelial cell loss while in storage as well as the length of storage time before losing clarity. Our results show that corneal ECD decreased steadily at a rate of 93.08 cells per mm^2 per day of storage. By extrapolation, the ECD would be expected to drop below the critical level of 2 200 cells per mm^2 after the 10^{th} post-harvest day. This is consistent with the finding that corneas preserved in Optisol-GS maintain their clarity only until the 9^{th} post-harvest day.

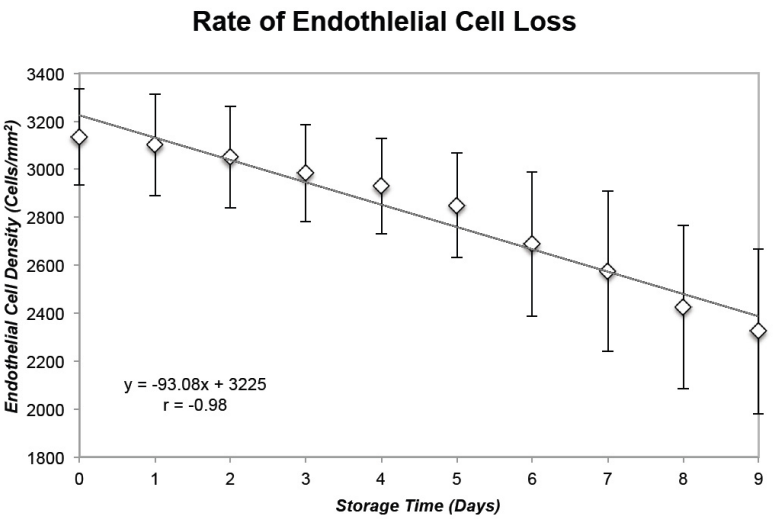

Figure1. Rate of endothelial cell loss

4. Conclusion

Although the ECD of corneas stored in Optisol-GS decreased steadily, it remained above the critical level throughout the 9-day observation period. This suggests that corneal buttons may remain viable for at least this amount of time. By the 10th post-harvest day, corneas began to lose their clarity. After the 10th post-harvest day the ECD would be expected to drop below the critical level. It would be a good practice to re-evaluate tissue stored in Optisol-GS and repeat specular microscopy prior to using the tissue if stored for more than 9 d.

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